Palliative Effect of Synchrotron Microbeam Radiation Therapy on EMT-6.5 and 67NR Tumours in a Mouse Model: Cellular Response of Normal and Tumour Tissue

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The aim of this study was to demonstrate a palliative effect of MRT in two different mouse tumour models, and then describe the short-term cellular response to MRT in normal and tumour tissues.

All irradiations were performed at the SPring-8, synchrotron in Japan using a polychromatic x-ray beam with a median energy of 110 keV. The beam was segmented into an array of 30 micron microbeams with peak-to-peak separations of 200 microns using a fixed collimator. BALB/c mice were inoculated subdermally on the right hind leg with EMT-6.5 or 67NR tumour cells and irradiated 6-9 days later with either 2 x 280 Gy or 2 x 560 Gy peak, in-beam air doses of MRT. The beams were delivered in a cross-hatched fashion, rotating the mouse 90o between the first and second irradiation. Mice were culled when the maximum length of the tumour reached 11 mm. Kaplan-Meier survival analyses were performed with log-rank, Breslow and Tarone-Ware significance tests. $P<0.05$ was considered significant. In other MRT experiments dorsal skinflaps and EMT-6.5 tumours were irradiated and tissues collected for immunohistochemical studies at different time points over the following 5 days.

The median survival times for EMT-6.5 and 67NR tumour bearing mice following MRT (2 x 560 Gy) increased from 16 to 29 and 27 to 42 days respectively compared to unirradiated controls ($P< 0.0005$). Immunohistochemistry for phosphorylated γH2AX (a surrogate marker for DNA DSBs) demonstrated nuclear localisation within cells situated in zones through the tissues that correlated well with microbeam spacing. γH2AX-positive cells were readily detectable from 6hrs to at least 4 days post MRT, suggesting that cells receiving very high levels of radiation fail to repair all DNA double breaks.

Double immunohistochemical staining with antibodies to γH2AX in conjunction with CD31, PCNA, CD45 and other markers has provided insights into normal tissue response to MRT, spatially resolved at a level identifying cells from peak and valley radiation exposure sites. Within 24 hours of MRT there is evidence of cell migration both into, and out from, irradiated zones. Irradiated endothelial cells were still present up to 4 days post treatment.

KEYWORDS: microbeam radiation therapy, tumour response, immunohistochemistry